

# Growth Hormone Deficiency Associated in the 18q Deletion Syndrome

Patricia Davis Ghidoni,<sup>1\*</sup> Daniel E. Hale,<sup>1</sup> Jannine D. Cody,<sup>2</sup> Charles T. Gay,<sup>1</sup> Nora M. Thompson,<sup>3</sup> Erin B. McClure,<sup>3</sup> Mark M. Danney,<sup>1,4</sup> Robin J. Leach,<sup>1,2</sup> and Celia I. Kaye<sup>1,2</sup>

<sup>1</sup>Department of Pediatrics, The University of Texas Health Science Center at San Antonio, San Antonio

<sup>2</sup>Department of Cellular and Structural Biology, The University of Texas Health Science Center at San Antonio, San Antonio

<sup>3</sup>Department of Psychiatry, The University of Texas Health Science Center at San Antonio, San Antonio

<sup>4</sup>Santa Rosa Children's Hospital, San Antonio, Texas

The 18q- syndrome is one of the commonest deletion syndromes. Clinical characteristics are variable but may include: hypotonia, tapered digits, "carp-like" mouth, mental retardation, and hearing impairment. Growth failure (GF; both weight and height <3%) was reported in 80% of affected individuals. We evaluated growth hormone (GH) sufficiency in 5 18q- syndrome patients, 3 of whom had growth failure (<3% weight and height); the remaining 2 had normal growth parameters. Laboratory evaluation of growth included measurement of IGF-1, IGFBP-3, bone ages and GH response to pituitary provocative agents. Three patients failed to produce adequate GH following stimulation testing. Of 3 patients with inadequate GH production, 1 had normal growth (above 3%). Only 1 of 5 patients had normal GH production and normal growth parameters. Our findings to date suggest that GH deficiency is common in individuals with the 18q- syndrome. The pathogenesis of this finding is unknown. We postulate that a gene(s) on 18q is involved in GH production. *Am. J. Med. Genet.* 69:7–12, 1997.

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**KEY WORDS:** growth hormone deficiency; 18q deletion syndrome; chromosome 18

## INTRODUCTION

The 18q deletion (18q-) syndrome, caused by a terminal deletion of the long arm of human chromosome 18, was originally identified by de Grouchy et al. in

1964. The phenotype is highly variable, but is characterized by mental retardation, short stature, hypotonia, hearing impairment, and foot deformities. Eighty percent of individuals affected with the 18q- syndrome have been reported to be below the fifth centile in height [Wilson et al., 1979]. Two affected individuals have been reported to be growth hormone-deficient [Schwarz and Duck, 1990; Andler et al., 1992].

The investigation of short stature has not traditionally been pursued in children with genetic syndromes. Poor growth was presumed to be multifactorial including the intrinsic cellular effects of aneusomy as well as intrauterine growth retardation. Classic growth hormone (GH) deficiency has no universally accepted criteria for diagnosis. Usual criteria include a combination of the following: height <3%, normal GH response to provocative stimulation, abnormal serum growth factor levels, abnormal height velocities and delayed bone age. GH has been widely available since 1985, leading to the possible treatment of partial- and non-GH deficient short children.

We have evaluated 5 patients with the 18q- syndrome for clinical manifestations including growth parameters and growth hormone response to provocative agents. We investigate what role growth hormone may play in their growth failure.

## MATERIALS AND METHODS

### Patient Population

Patients with deletions of chromosome 18q were referred from the Chromosome 18 Registry and Research Society, which is a support group for families with individuals with chromosome 18 abnormalities. Informed consent was obtained from all enrollees. Treatment with growth hormone, if deficiency was identified, was not contingent on participation in this study. The initial enrollees in the study were selected based on the proximity of their place of residence to our center. They were all females. Criteria such as short stature, growth velocity and cognitive function were not used in the selection of patients for evaluation. Height, weight and head circumference were measured on all subjects, and compared with age appropriate norms from National Center for Health Statistics [Hamill et al., 1979].

\*Correspondence to: Patricia Davis Ghidoni, M.D., UTHSCSA/Department of Pediatrics, 7703 Floyd Curl Dr., San Antonio, TX 78284-7809.

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### Molecular and Cytogenetic Analysis

Cytogenetic studies of all patients were performed by the referring institutions. Confirmation of the loss of material from the long arm of chromosome 18 was done using molecular analysis. High molecular weight DNA was isolated from peripheral blood leukocytes of the patient and both parents [Bell et al., 1981]. The analysis was performed using the polymerase chain reaction (PCR) first by end-labeling one primer of the set at the 5' end with  $\gamma$ - $^{32}$ P-dATP. PCR was then performed in a 10  $\mu$ l reaction volume containing 50 ng of genomic DNA, 50 ng of each primer, 200  $\mu$ M dNTPs, 1.5 mM MgCl<sub>2</sub> and 0.5 U *Taq* polymerase. The PCR reaction consisted of 5 minutes at 94°C followed by 30 cycles of 1 minute at 94°C, 1 minute at the optimal anneal temperature, 1 minute at 72°C, followed by 5 minutes at 72°C. PCR products were then separated on a 7% polyacrylamide gel run at 65 watts for 4 hours. Autoradiography was performed using Kodak XAR-5 film.

DNA was analyzed with 15 highly polymorphic microsatellite markers which had been identified previously and placed in linear order [Gyapay et al., 1994]. The polymorphic nature of these markers allows direct DNA analysis for one or two alleles at a specific locus. These markers are informative when the patient has two different allele sizes or when a unique allele from one parent is not present in the child. The results from a subset of the markers are shown in Figure 1.

### Growth Evaluation

Three separate measurements of height were obtained using a wall-mounted stadiometer. The reported height was the mean of these three measurements. Growth velocity was calculated based on the change in height over a 3 to 6 month interval. Bone maturity was determined by a radiograph of the wrist and hand using the standards of Greulich and Pyle [1959].

Growth promotion substances measured by The Nichols Institute were GH, insulin-like growth factor binding protein number 3 (IGFBP-3), insulin-like growth factor (IGF-1), and growth hormone binding protein (GHBP).

Clonidine stimulation for serum GH response was performed with the patient at complete rest (Fig. 2). Clonidine HCL was administered orally as a single dose of 5  $\mu$ g/kg of body weight. Blood samples for determination of GH levels were drawn at baseline (just before clonidine administration), 30, 90, and 120 minutes after the clonidine was administered. Serum and plasma samples were stored at -20°C until assayed. Specific radioimmunoassay techniques were used for determination of IGF-1 and IGFBP-3. GH levels were measured by immunochemiluminescence. GHBP was determined using ligand-mediated immunofunctional assay.

## CLINICAL REPORTS

### Patient 1

The female infant was evaluated for growth hormone status at the age of 12 months. She was born at 36 weeks of gestation with a weight of 2.6 kg and was noted to have epicanthal folds, foot deformity and hypotonia on the basis of a 46,XX, del(18)(q22) karyotype. Problems during infancy included recurrent vomiting and poor weight gain. Severe gastroesophageal reflux

and a large hiatus hernia were detected. After gastrostomy tube placement and Nissen fundoplication, growth and weight gain remained inadequate. A vertical talus was repaired at 6 months. Moderate hearing loss was diagnosed in infancy.

At age 12 months, psychological testing using the Bayley Infant Scales demonstrated standard scores of <50 on mental and motor developmental scales. Assessment of adaptive skills using the Vineland Scales documented age equivalents of 8 months in communication skills, 12 months in daily living skills, 12 months in socialization, and 7 months in motor abilities.

Growth parameters at 12 months included a length of 69 cm (<3rd centile for age, and at the 50th centile for a 6-month-old), weight of 7.5 kg (<3rd centile for age, and at the 50th centile for a 7-month-old), and head circumference (OFC) of 43 cm (2nd centile for age). Bone age was 9 months (1.7 S.D. below the mean for age) at 13 months.

### Patient 2

This woman was evaluated for growth hormone status at age 22 years. She was born at term with a birth weight of 3.1 kg, minor anomalies and a heart murmur. She walked at 18 months. Cleft palate and pulmonic stenosis were diagnosed during childhood. In her preschool years, severe language and fine motor delays were noted. A hearing loss of 30–40 dB was detected, and hearing aids were worn from years 3–13. Developmental testing of her nonverbal intelligence at 3 years 8 months suggested a mental age of 3 years, 5 months (DQ of 92). Testing at 6 years estimated her IQ to be 75.

At age 7 years, intravenous pyelography and cystoscopy were performed to evaluate enuresis, and a single hypertrophied left kidney was identified. Echocardiogram showed mild pulmonary and minimal mitral valve regurgitation.

Recurrent episodes of abdominal pain and vomiting began at 14 years. Development of breasts and pubic hair were at Tanner stage 4. Gynecologic evaluation for recurrent abdominal pain demonstrated Müllerian dysgenesis (blind uterine horns, absence of vagina/cervix, and uterine remnant) and severe endometriosis. Karyotype was 46,XX,del(18)(q23). At 22 years, her height (151.7 cm) and weight (44.6 kg) were at the 50th centile for a 12-year-old girl. She also had a short neck, sternal ear canals and a flat midface.

### Patient 3

This girl was evaluated at age 4 years. She was born at term by cesarean section because of placenta previa, with a birth weight of 3.6 kg. Karyotype was a 45,XX,del(18)(q22). She had recurrent otitis media in infancy; tympanotomy tubes were placed at 3 years. She was hospitalized at 2.5 years for pneumonia and bronchospasm.

This patient walked at 2.5 years and said "mama" at 2 years. At 5 years, developmental status was at an age equivalent of 42 months in cognitive skills, 21 months in expressive language, 42 months in receptive language and fine motor skills, and 33 months in gross motor skills.

When evaluated at 4 years, height (107.7 cm) and weight (18 kg) were at the 50th centile for age. OFC was

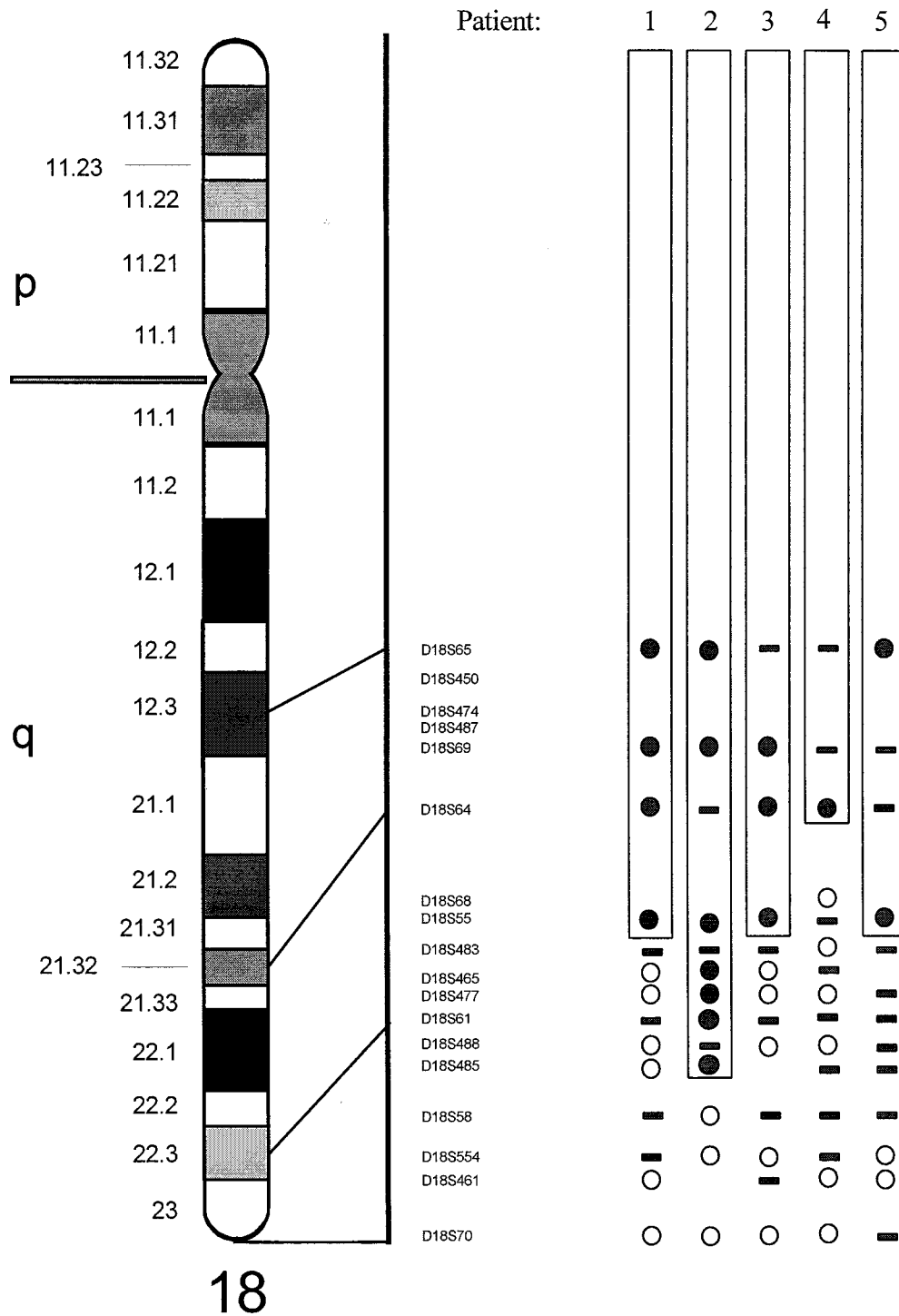


Fig. 1. Molecular analysis confirming the loss of chromosome material from 18q. DNA from each family was analyzed using the highly polymorphic markers developed and placed in a linear order by Génethon [Gyapay et al., 1994]. Black circles represent those markers for which the patient had 2 alleles, open circles represent those markers for which the patient had one allele and the dashes are markers which were uninformative. The rectangle indicates the minimal estimated size of the chromosome with the deletion.

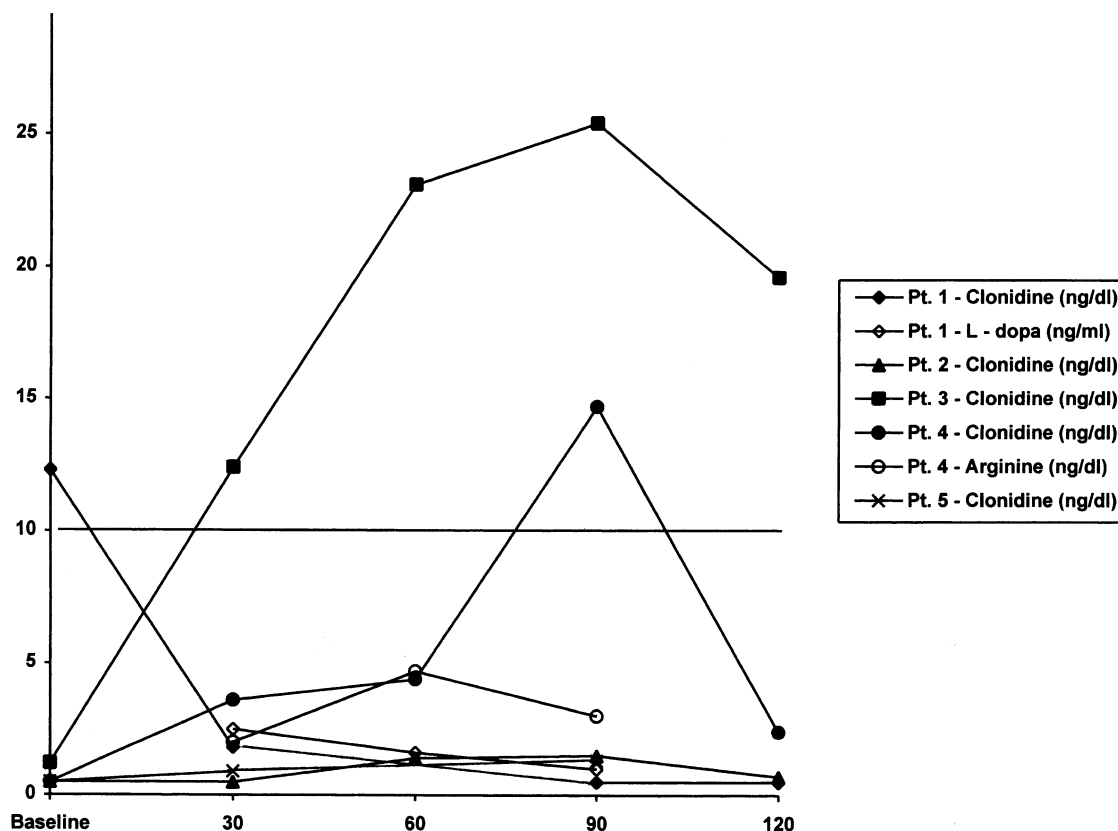


Fig. 2. Growth hormone production by patients in response to provocative agents. The horizontal line shown at 10 ng/dl is considered the normal response level.

49 cm (20th centile). She also had a wide mouth, simple helix, plump finger pads, and hyperextensible joints.

#### Patient 4

This girl was evaluated for growth hormone status at age 3 years. She was born spontaneously at term with oligohydramnios. Birth weight was 2.8 kg. Neonatally she had transient tachypnea. An echocardiogram showed pulmonary valvular stenosis and a muscular ventriculoseptal defect. She was hospitalized at 6 months for probably urinary tract infection and at 8 months for respiratory syn virus (RSV) bronchiolitis. She was readmitted two weeks later for pneumococcal sepsis and failure to thrive. Karyotype was 46,XX,del(18)(q21.3). At 3 years, developmental equivalents were 17 months in receptive language, 16 months in expressive language, 22 months in cognitive abilities and 12 months in gross motor skills. Her bone age was 18 months (2.7 S.D. below the mean).

At 35 months, her height (82.1 cm) and weight (9.2 kg) were below the 3rd centile for age. Her OFC was 49 cm (25th centile). She also had a broad nasal bridge, high arched palate, overlapping toes, and mild right clubfoot deformity.

#### Patient 5

This girl was evaluated for growth hormone status at age 3 years. She was born at term weighing 3.4 kg; preg-

nancy and delivery were uncomplicated. At 4 months, a murmur was detected and echocardiogram demonstrated anomalous pulmonary venous return. Karyotype was 46,XX,del(18)(q22.2). Cardiac surgery was performed at 5 months. Tympanotomy tubes were placed at 26 months for multiple ear infections. Bilateral grade III vesicoureteral reflux and a small left kidney were detected on the voiding cystourethrogram and renal ultrasound studies following urinary tract infection.

At 2 years, the patient could not walk without assistance. Her vocabulary consisted of 10–20 words. At 3 years, developmental equivalents were 27 months for cognitive skills, receptive language of 20 months, expressive language of 18 months, fine motor skills of 17 months and gross motor skills of 14 months.

At 3 years, her height was 94.5 cm (50th–75th centile for age), weight 13.2 kg (25th centile), and OFC 48 cm (10th centile for age). She also had epicanthal folds, broad nasal bridge, prominent forehead, wide mouth, clinodactyly of fifth digits, hypotonia, and hyperextensible joints.

## RESULTS

Molecular genetic analysis confirmed the diagnosis of a deletion of the long arm of chromosome 18 in all subjects (Fig. 1). Growth status and bone age (where available) of each subject are shown in Table I.

Recently it has become evident that there is a spectrum of GH production ranging from normal to com-

pletely deficient. The ability to identify a GH production abnormality depends on the particular test used (e.g., provocative test versus 12 hour blood collection), the clinical status of the child (e.g., well-nourished versus malnourished), and the probable site of the defect within the central nervous system (e.g., pituitary versus hypothalamus). For this reason, the following criteria were used in classifying our patients as GH-deficient, GH-insufficient or normal. Normal individuals had a height consistent with familial heights, a growth rate within 2 S.D. for age, normal bone age and a normal GH peak in response to a provocative agent. GH-deficient children had a length or height inconsistent with parental heights, abnormal growth rates, delayed bone age and a GH peak response to clonidine of less than 7 ng/dl. GH levels between 7 and 10 ng/dl were considered borderline. The use of several provocative tests to assess GH reserve can improve specificity. We were limited in obtaining a second provocative test in 3 of our study patients by constraints of time and patient comfort. All patients had at least one clonidine test, a simple, safe and reliable test. GH-insufficient individuals were those not fitting the criteria of either category. Bone age was obtained from hand and wrist radiographs according to the standards of Greulich and Pyle [1959].

Based on these criteria, only Patient 3 was unequivocally normal. Her height was consistent with parental heights, her growth rate was appropriate for age, growth factors were within the normal range for age and her peak GH response to stimulation was normal. Patient 5 was at the 60th centile for age with a normal growth velocity; however, her peak response to clonidine was only 1.3 ng/dl.

The other 3 individuals have findings of growth hormone insufficiency or deficiency. Patients 1 and 4 had a height inconsistent with parental heights and below the 5th centile for age. Both had delayed bone maturation and abnormal linear growth velocity. Patient 4 had a normal response to clonidine but failed to respond to arginine. Patient 1 had a low normal response to clonidine and failed to respond to L-dopa as a second provocative agent. The mature patient 2 was <3% in height. Her parents were of average height. By report, she had gone through puberty at 13 years. She failed to respond to clonidine.

Growth factors (IGF-1, IGF-1, GHBP) were not useful in differentiating children with growth failure or GH deficiency from those growing normally, since all of the results were within the range of normal for age

(Table II). These substances are mediators for growth promotion or their receptors. GHBP is the peptide that binds GH in the blood. IGF-1 is a protein that binds and modulates the action of IGF-1. IGF-1 is a peptide that is active in stimulating the proliferation of cartilage fibroblasts and other tissues. Although IGF-1 can be lowered in states of chronic disease and malnutrition, this was not a consideration in these patients as none had low IGF-1 levels. The normal IGF-1 values suggest that the 5 subjects were in reasonable nutritional balance as well and that nutrition did not contribute to their linear growth problems.

Several other anomalies were observed in our patients and were reported previously in the 18q- syndrome and include low IgA, cardiac anomalies, orthopedic problems, and urinary tract abnormalities (Table III).

## DISCUSSION

Poor growth is a common but not universal finding in individuals with deletions of 18q. Reports of GH deficiency in two patients with 18q- syndrome prompted us to search for this abnormality in additional patients. In our study of 5 18q- individuals, 3 exhibited deficient growth (patients 1, 2 and 4) and all 3 demonstrated growth hormone deficiency or insufficiency. Thus, it is likely that growth hormone insufficiency is a major factor leading to growth failure in individuals with this disorder. However, growth hormone insufficiency is not found in all 18q- subjects.

Failure in patient 5 to respond to clonidine, who was growing normally, is not unique. Depending on the medication used, 5% to 15% of children who are growing will fail to respond to a single provocative test [Lanes et al., 1985]. This has been the reason for using at least 2 provocative agents prior to identifying a child as GH-deficient. Normal growth has been reported in some children after cranial irradiation, despite abnormalities in the secretion of growth hormone [Jorgensen et al., 1993]. This observation suggests that some children with subnormal growth hormone response grow normally.

Growth hormone treatment of individuals with 18q- syndrome was reported by Schwarz and Duck [1990] and Andler et al. [1992]. Both of these individuals responded to growth hormone therapy with improved growth velocity. We treated patient 1 with synthetic growth hormone 2.5 mg 3 times weekly with improved growth velocity (34.3 cm/yr from 15.2 cm/yr) over a period of 12 months. Patient 4 is now receiving growth hormone; growth and growth velocity data are not yet available.

TABLE I. Growth Parameters of 18q- Study Patients

PT	Lab no.	Age	Height (cm)	Height centile	Height Z score	Bone age (S.D.)	Growth velocity (GV) (cm/yr)	GV centile	Clonidine GH peak ng/dl	Other GH peak ng/dl
1	2	13 mo	66.0	<5	-2.1	-1.7	17.2	50	12.3	2.5
2	1	22 yrs	151.7	<5	-2.1		0.95	25	1.5	
3	5	57 mo	103.5	40	-0.5		6.77	50	25.4	
4	9	33 mo	82.1	<5	-2.2	-2.7	0.5	10	14.7	4.7
5	7	33 mo	97.2	60	+0.4		5.6	50	1.3	

TABLE II. Growth Factors in Patients With 18q-\*

	Patient no.				
	1	2	3	4	5
IGF-1 (ng/ml)	47 (17-248)	213 (182-780)	62 (17-248)	91 (17-248)	224 (17-248)
IGFBP-3 (mg/L)	0.9 (0.7-2.3)	2.2 (2.0-4.1)	1.7 (0.9-4.1)	2.1 (0.9-4.1)	1.7 (0.9-4.1)
GHBP (pmol/L)	94 No RR	224 No RR	165 (65-393)	158 No RR	124 No RR

\*RR, reference range.

TABLE III. Clinical Findings in Patients With 18q-

	Patient number					Frequency in other patients [Wilson et al., 1979] (%)
	1	2	3	4	5	
Cognitive language delay	+	-	+	+	+	95
Hypoplastic midface	+	+	+	+	+	85
"Carp-like" mouth	+	-	+	+	+	87
Short stature (<5%)	+	+	+	-	-	80
Hypotonia	+	-	+	-	+	91
Congenital heart disease	-	+	+	-	+	38
Hearing impairment	+	+	-	-	-	57
Renal anomalies	-	+	-	-	+	ND <sup>a</sup>
Foot anomalies	+	-	+	+	-	25
Hyperextensible joints	-	-	+	-	+	ND
IgA deficiency	-	-	+	-	+	35
Multiple infections	-	-	+	+	+	ND
Müllerian agenesis	-	+	-	-	-	ND

<sup>a</sup> ND, condition not described in other publications.

The cause of growth hormone insufficiency, and associated growth failure, in 18q- children is not yet apparent. None of the characterized growth promoting substances or receptors has been localized to 18q. The growth hormone gene was mapped to 17, and the gene for growth hormone releasing hormone to 20 [Le Beau and Geurts vanKessel, 1991]. Data presented here suggest that a gene or genes important for growth hormone production or release is located on 18q.

Experience with patient 1, and 2 individuals reported by others, suggests that children with 18q- syndrome and growth hormone insufficiency may respond to growth hormone. Since patient 1 and the previously reported subjects have not yet reached puberty, improvement in adult height can not be assumed. However, our findings suggest that subjects with 18q- syndrome and growth failure should be evaluated for GH insufficiency and that growth hormone treatment should be considered in children who meet appropriate clinical criteria.

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